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PATENT
Atty. Docket No. EXT-073 C1
(2457/71)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS:	Boles et al.		
SERIAL NUMBER:	Continuation of 09/336,609	GROUP NO.:	Not yet assigned
FILING DATE:	Herewith (December 19, 2001)	EXAMINER:	Not yet assigned
TITLE:	Detection of Non-Viral Organisms With SRP RNA		

Box PATENT APPLICATION
Commissioner for Patents
Washington, D.C. 20231

Sir:

PRELIMINARY AMENDMENT

AMENDMENTS

Prior to examination, please enter the following Amendments to the above-identified application.

IN THE SPECIFICATION:

Please enter the sequence listing. Please further amend the specification to read as follows. A marked-up copy of the amended paragraphs showing the amendments is attached.

On the first page of the specification, please amend the second line starting with Attorney's Docket No. to recite:

Attorney's Docket No. EXT-073 C1

On the first page of the specification under CROSS REFERENCE TO RELATED APPLICATIONS, please amend the first paragraph to recite:

The present application is a continuation of U.S. Patent Application Serial No. 09/336,609, filed June 18, 1999, which claims priority to U.S. Provisional Application Serial No. 60/090,063, filed June 19, 1998 and is related to U.S. Patent Application Serial No. 08/971,845, filed August 8, 1997; the entire disclosures of which are incorporated herein by reference.

On the 26th page of the specification under Sequences of Oligonucleotides used, please amend the first full paragraph to recite:

4.5S probe (2nf): GGCACACGCGTCATCTGC (SEQ ID NO:9)
5S probe (66ng): CCACACTACCATCGGCGCT (SEQ ID NO:20)

On the 28th page of the specification, please amend the first full paragraph to recite:

Aliquots were thawed, 20% sodium dodecyl sulfate was added to a final concentration of 1.4% in a total volume of 15.6 µl, and tubes were heated at 130°C for 10 minutes. Tubes were removed to room temperature for several minutes, and hybridization mix was added to a final volume of 20 µl with the following final concentrations: 120 mM NaCl, 1 mM $MgCl_2$, 0.1 mM $ZnCl_2$, 22.5 mM Tris (pH 8), 22.5 mM boric acid, 0.5 mM aurin tricarboxylic acid, 8mM Na phosphate, and 50 nM of each of the alkaline phosphate-conjugated reporter probes, RP-1 (5'-alkaline phosphatase-GCUGCUUCCUUC (SEQ ID NO: 27); underlined bases represent 2'-O-methyl RNA nucleotides) and RP-2 (5'-alkaline phosphatase-GCUGCUUCCGUC (SEQ ID NO:14). These mixtures were warmed to 55°C for 10 minutes, then removed to room temperature and 4µl of loading buffer (50% glycerol, 0.2% xylene cyanole, 0.2% bromphenol blue) added. Half of each mixture was loaded onto a 5% polyacrylamide gel (89 mM Tris (pH 8.5), 27 mM phosphate buffer), made with 10µM of each of the following five acrydite-modified, 2'-O-methyl RNA capture probes, polymerized into the gel in a fashion similar to that described in Example III.

CP-1 5'-acrydite-TTTTTT-CGGACCUGACCUG (SEQ ID NO:15)
CP-2 5'-acrydite-TTTTTT-AGGACCUGACAUG (SEQ ID NO:16)
CP-3 5'-acrydite-TTTTTT-CGGACCUGACCAG (SEQ ID NO:17)
CP-4 5'-acrydite-TTTTTT-CGGACCUGACAAG (SEQ ID NO:18)
CP-5 5'-acrydite-TTTTTT-CGGAUCUGACACG (SEQ ID NO:19)

The gel was run at 30°C at 20 volts/cm for 30 minutes, rinsed in diethanolamine buffer (2.4 M diethanolamine, 1 mM $MgCl_2$, 0.1 mM $ZnCl_2$, pH 10) for 10 minutes, then AttoPhos™ chemiflourescent substrate (Boehringer-Mannheim) was added for 10 minutes. The reaction was

stopped by the addition of 1M Na phosphate (pH 7.2) and the fluorescent signal was scanned on a Molecular Dynamics Fluorimager 595 (*see* Figure 7). All nine of the listed bacterial species were detected with this probe set.

IN THE CLAIMS:

Please cancel claims 6, 7, 9, 13-18, 21-23, 25, 27, 28, 31-39, 41 and 45-49 without prejudice.

Please amend claim 19 to recite:

19. (Amended) The method of claim 1, wherein the nucleic acid probe has a nucleotide sequence selected from the group consisting of: GCTGCTTCCTTCCGGACCTGAC (SEQ ID NO:2); GCTGCTTCCTTCCGGACCTGA (SEQ ID NO:3); GGCACACGCGTCATCTGC (SEQ ID NO:9); GCTGCTTCCTTC (SEQ ID NO:4); GCTGCTTCCTTCCGGACCTGAGTGAATACGTTCCCGGGCCT (SEQ ID NO:7); GCTGCTTCCTTCCGGACCTGACAAAAACGATAAACCAACCA (SEQ ID NO:8); GCTGCTTCCTTCCGGACCTGACCTGGTAAA (SEQ ID NO:11); GCTGCTTCCTTCCG (SEQ ID NO:5); GACCTGACCTGGTA (SEQ ID NO:6); GCTGCTTCCGTC (SEQ ID NO: 21); CGGACCTGACCTG (SEQ ID NO:22); AGGACCUGACAUG (SEQ ID NO:23); CGGACCUGACCAG (SEQ ID NO:24); CGGACCUGACAAG (SEQ ID NO:25); and CGGAUCUGACACG (SEQ ID NO:26).

Please amend claim 35 to recite

35. (Amended) The method of claim 20, wherein the gel-immobilized nucleic acid probe has the nucleotide sequence selected from the group consisting of: GCTGCTTCCTTCCGGACCTGAC (SEQ ID NO:2); GCTGCTTCCTTCCGGACCTGA (SEQ ID NO:3); GGCACACGCGTCATCTGC (SEQ ID NO:9); GCTGCTTCCTTC (SEQ ID NO: 4); GCTGCTTCCTTCCGGACCTGACCTGGTAAA (SEQ ID NO:11); GCTGCTTCCTTCCG (SEQ ID NO:5); GACCTGACCTGGTA (SEQ ID NO:6); GCTGCTTCCGTC (SEQ ID NO:21); CGGACCTGACCTG (SEQ ID NO:22); AGGACCUGACAUG (SEQ ID NO:23); CGGACCUGACCAG (SEQ ID NO:24); CGGACCUGACAAG (SEQ ID NO:25); and CGGAUCUGACACG (SEQ ID NO:26).

REMARKS

After entry of this Preliminary Amendment, claims 1-5, 8, 10-12, 19, 20, 24, 26, 29, 30, 40, 42-44 and 50 are pending in the Application. Applicants have canceled claims 6, 7, 9, 13-18, 21-23, 25, 27, 28, 31-39, 41 and 45-49 without prejudice and without any intention to abandon

the subject matter as filed, but with the intention that claims of the same, greater, or lesser scope may be pursued in this or a continuing application. Applicants respectfully submit that no new matter is introduced by the amendment. The Specification is herein amended to recite related applications and to incorporate the sequence listing and appropriate sequence identifiers. Applicants respectfully submit that the aforementioned amendments introduce no new matter.

CONCLUSIONS

Applicants respectfully request entry of this amendment prior to examination of the application on the merits. The Examiner is invited to telephone Applicants' undersigned representative to discuss any outstanding issues.

Applicants believe that no fees are due with this submission. However, the Director is hereby authorized to charge any fees that may be due to deposit account No. 20-0531.

Respectfully submitted,

Date: December 19, 2001
Reg. No. 43,526
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MARKED UP SPECIFICATION SHOWING AMENDMENTS

Second Line on Page 1:

Attorney's Docket No. [018422-000210] EXT-073 C1

Cross Reference to Related Applications on Page 1:

CROSS REFERENCE TO RELATED APPLICATIONS

The present application is a continuation of U.S. Patent Application Serial No. 09/336,609, filed June 18, 1999, which [The present application] claims priority to U.S. [Patent] Provisional Application Serial No. 60/090,063, filed June 19, 1998 and is related to U.S. Patent Application Serial No. 08/971,845, filed August 8, 1997, [herein both incorporated by reference.]; the entire disclosures of which are incorporated herein by reference.

First full paragraph on page 26:

Sequences of Oligonucleotides used:

4.5S probe (2nf): GGCACACGCGTCATCTGC (SEQ ID NO:[8]9)
5S probe (66ng): CCACACTACCATCGGCGCT (SEQ ID NO:[9]20)

First full paragraph on page 28:

Aliquots were thawed, 20% sodium dodecyl sulfate was added to a final concentration of 1.4% in a total volume of 15.6 µl, and tubes were heated at 130°C for 10 minutes. Tubes were removed to room temperature for several minutes, and hybridization mix was added to a final volume of 20 µl with the following final concentrations: 120 mM NaCl, 1 mM $MgCl_2$, 0.1 mM $ZnCl_2$, 22.5 mM Tris (pH 8), 22.5 mM boric acid, 0.5 mM aurin tricarboxylic acid, 8mM Na phosphate, and 50 nM of each of the alkaline phosphate-conjugated reporter probes, RP-1 (5'-alkaline phosphatase-GCUGCUUCCUUC (SEQ ID NO:[4]27); underlined bases represent 2'-O-methyl RNA nucleotides) and RP-2 (5'-alkaline phosphatase-GCUGCUUCCGUC (SEQ ID NO:14). These mixtures were warmed to 55°C for 10 minutes, then removed to room temperature and 4 µl of loading buffer (50% glycerol, 0.2% xylene cyanole, 0.2% bromphenol blue) added. Half of each mixture was loaded onto a 5% polyacrylamide gel (89 mM Tris (pH 8.5), 27 mM phosphate buffer), made with 10 µM of each of the following five acrydite-modified, 2'-O-methyl RNA capture probes, polymerized into the gel in a fashion similar to that described in Example III.

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CP-5 5'-acrydite-TTTTTT-CGGAUCUGACACG (SEQ ID NO:19)

The gel was run at 30° at 20 volts/cm for 30 minutes, rinsed in diethanolamine buffer (2.4 M diethanolamine, 1 mM $MgCl_2$, 0.1 mM $ZnCl_2$, pH 10) for 10 minutes, then AttoPhos™ chemiflourescent substrate (Boehringer-Mannheim) was added for 10 minutes. The reaction was stopped by the addition of 1M Na phosphate (pH 7.2) and the fluorescent signal was scanned on a Molecular Dynamics Fluorimager 595 (*see* Figure 7). All nine of the listed bacterial species were detected with this probe set.

MARKED UP CLAIMS SHOWING AMENDMENTS

Claim 19:

19. (Amended) The method of claim 1, wherein the nucleic acid probe has a nucleotide sequence selected from the group consisting of: GCTGCTTCCTTCCGGACCTGAC (SEQ ID NO:2); GCTGCTTCCTTCCGGACCTGA (SEQ ID NO:3); GGCACACGCGTCATCTGC (SEQ ID NO:9); GCTGCTTCCTTC (SEQ ID NO:4); GCTGCTTCCTTCCGGACCTGAGTGAATACGTTCCCGGGCCT (SEQ ID NO:7); GCTGCTTCCTTCCGGACCTGACAAAAACGATAAACCAACCA (SEQ ID NO:8); GCTGCTTCCTTCCGGACCTGACCTGGTAAA (SEQ ID NO:11); GCTGCTTCCTTCCG (SEQ ID NO:5); GACCTGACCTGGTA (SEQ ID NO:6); GCTGCTTCCGTC (SEQ ID NO:[14]21); CGGACCTGACCTG (SEQ ID NO:[15]22); AGGACCUGACAUG (SEQ ID NO:[16]23); CGGACCUGACCAG (SEQ ID NO:[17]24); CGGACCUGACAAG (SEQ ID NO:[18]25); and CGGAUCUGACACG (SEQ ID NO:[19]26).

Claim 41:

41. (Amended) The method of claim 20, wherein the gel-immobilized nucleic acid probe has the nucleotide sequence selected from the group consisting of: GCTGCTTCCTTCCGGACCTGAC (SEQ ID NO:2); GCTGCTTCCTTCCGGACCTGA (SEQ ID NO:3); GGCACACGCGTCATCTGC (SEQ ID NO:9); GCTGCTTCCTTC (SEQ ID NO: 4); GCTGCTTCCTTCCGGACCTGACCTGGTAAA (SEQ ID NO:11); GCTGCTTCCTTCCG (SEQ ID NO:5); GACCTGACCTGGTA (SEQ ID NO:6); GCTGCTTCCGTC (SEQ ID NO:[14]21); CGGACCTGACCTG (SEQ ID NO:[15]22); AGGACCUGACAUG (SEQ ID NO:[16]23); CGGACCUGACCAG (SEQ ID NO:[17]24); CGGACCUGACAAG (SEQ ID NO:[18]25); and CGGAUCUGACACG (SEQ ID NO:[19]26).